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A Method to Detect the Carcinoemryonal Antigen with a Special Antibody Diagnosticum

[SPOSOB OBNARUZHENIYA RAKOVO-EMBRIONALNOGO ANTIGENA PRI POMOSHCHI SPETSIALNOGO ANTITELNOGO DIAGNOSTIKUMA]

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(54) A METHOD TO DETECT THE CARCINOEMRYONAL ANTIGEN WITH A SPECIAL ANTIBODY DIAGNOSTICUM

(57) **Abstract.** Application: in the field of medicine, especially, in immunology. Subject matter of the invention: Patient's blood serum samples are mixed with the diagnosticum obtained antibodies specific for CEA (carcinoemryonal antigen) related to blood erythrocytes of one or two-year-old chicken; the erythrocytes are treated with glutaraldehyde followed by registration of aggregate

hemagglutination of the antigens with antibodies. The method simplifies the detection of EARs (erythrocyte agglutination reactions) and may be widely allied to routine medical practice. 4 tables.

The invention concerns medicine, the methods of diagnostics, specifically to an immunoassay that is based on the preparations, which contain antigen-conjugated carriers and it may be applied for detection of patients having tumors, specifically, the producers of carcinoemryonal antigen (CEA). CEA, as a tumor antigen is a glycoprotein (molecular mass = 200,000 Daltons). Based on our own data and literature (1) the CEA may be detected in blood of healthy individuals in concentrations ranging from 0 to 20 ng/mg (if detected by a radio immune assay method); concentrations of CEA more than 40 ng/ml point to the presence of the malignant tumor growth in the human body.

The known method of radio immune assaying (RIA) is used for determination of CEA concentrations in humans. This method includes mixing of human blood serum with the specific radioisotope labeled antibodies, followed by incubation, separation, radiometry, and data recording (2).

This method provides the means for determination of CEA in blood serum of patients in concentrations ranging from 0 to 360 ng/ml and higher.

However, wide application of such a method, including mass health examinations in outpatient clinics, actually, is completely impossible in practice in this country due to the lack of proper chains of radio immune diagnostic laboratories; limited number and high costs of domestic and foreign-produced radio immune sets and rather expensive instrumentation for such examinations. Further, the presence of radioactivity requires proper protection and precautions for the procedure of protein labeling with radionuclides, the same as at preparation and completion of radio immune assays. There is a need in custom-designed premises,

facilities, means of personnel protection, specific methods of transportation and burial of radioactive waste.

Taking account of the above factors in their integrity, we got the stimulus to search for some other antibody carriers suitable for preparation of the antibody diagnosticums, which are used in the Ag-A (antigen-antibody) reactions.

The authors have completed a research project to investigate some other carriers (erythrocytes of rabbits, sheep, and birds: chicken and pigeons) having capacity to adsorb antibodies on their surfaces. Chicken erythrocytes have occurred to be the most suitable for the assaying purposes of all the types of erythrocytes mentioned. Based on application of the chicken erythrocytes we developed a low-cost and simple method of the CEA detection (taking account of the available everywhere crude material required to prepare the erythrocyte antibody diagnosticum.

In our method of CEA detection in humans the patient's blood serum is mixed with the specific erythrocyte-conjugated antibodies followed by registration of antigen-antibody interaction data in the Ag-A reaction. The antibody

erythrocyte diagnosticum is produced by treating the erythrocytes of 1 to 2-year-old chicken with glutaraldehyde and adding of the anti-CEA-antibodies to the erythrocytes.

The subject matter of our invention is that we have examined and selected the chicken erythrocytes, which show high absorbing capacity and minimum tendency to the cross reaction with human tissues.

The preparation of chicken blood, being on the by-products at the poultry processing plants, does not require any sterile facilities or other specific conditions mentioned for blood sampling in humans. The technology of chicken blood sampling results in a considerable cost reduction for one of the principal components (specifically, erythrocytes) of the Ag-A reaction.

All other stages at preparation of the reacting agents for the Ag-A reaction do not differ significantly from the prototype.

Thus, based on the application of chicken erythrocytes instead of ones of humans eliminates the risk for donors and

medical personnel to be infected with infectious agents; it makes easy the technology of reactant preparation and costs of the reaction.

Chicken erythrocytes are obtained by decapitation of birds followed by taking of their blood into the Olveser's medium in the 1:2 ratio. The blood is filtered, and the produced erythrocytes are washed off 10 times by a physiologic saline and centrifuged at 4,000 rev/min. Once double the volume of physiologic saline (pH = 7.2) containing 0.25 % solution of glutaraldehyde is added to the resulting erythrocyte sediment, the suspension is stirred and incubated within 3 hrs at 37 °C. Following to the incubation the erythrocytes are washed off 4 times with the physiology saline in the 1:10 ratio; this operation is followed by adding of physiology saline to the erythrocyte sediment to produce a 8 % suspension. Sodium merthiolate up to its 1:10,000 final concentration is added for preservation purposes.

To produce the diagnosticum for 40 determinations, one has to take 1 ml of CEA antibodies (pH = 7.2 - 7.4), then add 2.5 % aqueous solution of glutaraldehyde (0.03 - 0.04 ml) and incubate for 1 hr 37 °C.

Once 1 ml of glutarized CEA antibodies and 20 mg of sodium bicarbonate added to 1 ml of 8 % suspension of erythrocytes, the mixture is incubated within 2 hrs at 56 °C, and then for half an hour at room temperature and continuous shaking. The erythrocytes are washed off from unconjugated antibodies by 4 times centrifuging at 3,000 rev/min, 4 minutes each time. The sediment is used to prepare an 8 % suspension in physiologic saline. Just the suspension is the specific erythrocyte antibody diagnosticum.

Case Study for Method Realization

The reaction was completed in U-shaped polystyrene plates. Physiological saline is placed into the plate's holes (0.025 ml per a hole); dilutions of the patient's nonactivated blood serum are prepared starting from 1:2.

The erythrocyte diagnosticum is added into the holes, 0.025 ml each hole. The last, eighth hole in each row serves as a reference standard for the diagnosticum (no antigen, which is contained in the serum examined). The plate is shaken up

gently, and then placed into a drying cabinet at 37 °C. The result of this reaction is assessed 1.5 to 2 hrs after, and the reactions are counted by "ring" formation based on the 4-point system.

Based on the comparative examination of antibody erythrocyte diagnosticums in the Ag-A reaction at different concentrations of the CEA standard in physiologic saline it was shown that with the anti-CEA antibody diagnosticum made of chicken erythrocytes it was possible to detect at the aggregate hemagglutination reaction in human blood serum the specific antigen in concentrations up to 8 ng/ml; such a sensitivity compares well with the aggregate hemagglutination reaction at the diagnosticum of human erythrocytes (Table 1).

As follows from the data shown in Table 1, there was no difference in the sensitivity of the Ag-A reactions completed with chicken and human erythrocytes.

The data shown in Table 2 illustrate that (1) the titers of Ag-A reactions depend on the CEA concentrations in blood serum: the higher is a CEA concentration, the greater is the

titer of positive reaction; (2) the anti-CEA antibody diagnosticum of chicken erythrocytes is equal in its activity to that of human erythrocytes.

Thus, our determination of sensitivity of the anti-CEA antibody diagnosticums has revealed that the erythrocyte antibody diagnosticum (EAD) of chicken erythrocytes in its sensitivity is on a level with that of human erythrocytes and it allows detection of CEA at the Ag-A reaction in concentrations of 8 ng/ml or higher.

For the primary product (chicken erythrocyte) saving purposes we have experimentally selected the maximal of concentrations of erythrocytes in EADs, which secure reliable data for the Ag-A reactions.

A few dilutions (6 %; 5 %; 4 %; 3 % and 2 %) were made from the prepared EAD, which contained 8 % suspension of erythrocytes, and the Ag-A reaction completed with the CEA standard (477 ng/ml). The resulting data are shown in Table 3.

As follows from the data shown in Table 3, the highest number of positive results was obtained at application of 4 % EAD and at maximum CEA dilutions (1:64 - 1:128) corresponding to 7.5 - 3.8 ng/ml. In our further examinations for the Ag-A reaction we applied CEA containing 4 % of chicken erythrocytes.

To assess the accuracy of the Ag-A reaction with EAD of chicken erythrocytes we have completed examination of 25 reference standard CEA samples, blood serum of 30 donors, 30 cancer patients, and 186 patients having acute or chronic inflammatory diseases (Table 4).

The titer of the Ag-A reaction in healthy individuals was no more than 1:4. Nearly similar data were obtained at examination of non-cancer patients: in 183 patients of 186 in total (98.9 %) of this group the positive reactions were found for the 1:2 - 1:4 titers, and only for 3 patients (1.2 %) in the 1:8 titer. For our cancer patients the titers of Ag-A reaction in the overwhelming majority of cases (86.6 %) ranged from 1:8 to 1:64.

To summarize, application of the Ag-A reaction and EAD of chicken erythrocytes makes it possible to detect the patients with high titers of the reaction (i.e. high concentrations of CEA) in blood serum being indicative of the patients' possible cancers and pointing to the need of their further examination by the oncology specialists.

Based on all the above it is possible to suggest that the authors proposed a new method of CEA detection in blood serum of patients, which points to high probability of malignant neoplasms to be found in these individuals. The proposed method is principally new in that the inventors pioneered to apply glutarized chicken erythrocytes with the anti-CEA antibodies adsorbed on their surface for the CEA detection.

The invention essence of this method is that the inventors have used new pioneering immunology approaches to detection of CEAs; these approaches were realized based on adding of specific anti-CEA antibodies to a new, not applied before carrier, i.e. to the chicken erythrocytes.

The proposed method of CEA detection with the antibody diagnosticum based on chicken erythrocytes is simple; it also is characteristic of high availability of the raw product (chicken erythrocytes), safety of laboratory manipulations and low costs of the reactants applicable. The method is suitable for large-scale application in the routine medical practice.

The Claim

A method to detect the carcinoemryonal antigen (CEA) with a special antibody diagnosticum through mixing of blood serum with the diagnosticum produced of the CEA-specific antibodies, which are conjugated with the erythrocytes treated by glutaraldehyde; which is followed by recording of the aggregate hemagglutination reaction of the antigen and antibody distinctive in that as the diagnosticum were used the specific anti-CEA-antibodies bound with the glutaraldehyde-treated blood erythrocytes of one- and two-year-old chicken.

Table 1

Comparative determination of thresholds of sensitivity for the Ag-A reaction with antibody diagnosticums of chicken and human erythrocytes

##	Antibody erythrocytes of erythrocytes	Number of tests	Number of positive reactions							
			CEA dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128	Ref.
			CEA Concentration (ng/ml)	238	119	59	29	15	7.5	3.8	0
1	Humans	10		10	10	10	10	10	9	8	0
2	Chicken	10		10	10	10	10	10	9	8	0

Table 2

Comparative results of determination of presence of CEA with antibody diagnosticums of two types

#	Sample	Antibody diagnosticum of erythrocytes	Number of tests	Number of positive samples based on reaction titers					
1	Blood serum of healthy donors			1:2	1:4	1:8	1:16	1:32	1:64
		Humans	24	14	10				
		Chicken	42	28	14				
2	Blood (CEA at serum of RIA up to	Humans	10			2	8		
		Chicken	13			3	10		
3	Blood 20 ng/ml) serum of CEA at RIA from 0 to 40 ng/ml)	Humans	10			2	2	3	3
		Chicken	11			3	1	4	3

Table 3

The results Ag-A reactions referenced to the CEA reference standard (477 ng/mg) at different concentrations of chicken erythrocytes in EAD

#	Erythrocyte concentration in EAD (%)	Number of tests	Number of positive Ag-A reactions							
			CEA dilution	2:1	4:1	8:1	16:1	32:1	64:1	128	Ref
			CEA concentration (ng/mg)	238	119	59	29	15	7.5	3.8	0
1	8	4	4	4	4	4	4	4	3	3	-
2	6	4	4	4	4	4	4	4	3	3	-
3	5	4	4	4	4	4	4	4	3	3	-
4	4	4	4	4	4	4	4	4	4	4	-
5	3	4	4	4	4	4	4	4	3	-	-
6	2	4	4	4	4	4	4	3	-	-	-

Table 4

The results Ag-A reactions for blood serum of sick and healthy humans with EAD of chicken erythrocytes

#	Patients	Number of tests	Number of positive data at blood serum dilution:							
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	Ref.
1	Cancer patients	30	1	3		10	3	2	-	-
	Inflammatory disease patients	186	175	8	3	-	-	-	-	-
3	Healthy persons	30	23	7	-	-	-	-	-	-
4	CEA standard	5	5	-	-	-	-	-	-	

	15 mg/ml									
5	CEA standard 45 mg/ml	8	2	6	-	-	-	-	-	-
6	CEA standard 477 mg/ml	12	-	-	-	-	-	3	9	-